

FIGURE 1

(SEQ ID NO:1-nucleotide sequence and SEQ ID NO:2-amino acid sequence)
Escherichia coli B Phytase Sequence

1
 ATG AAA GCG ATC TTA ATC CCA TTT TTA TCT CTT CTG ATT CCG TTA ACC CCG
 Met Lys Ala Ile Leu Ile Pro Phe Leu Ser Leu Leu Ile Pro Leu Thr Pro
 CAA TCT GCA TTC GCT CAG AGT GAG CCG GAG CTG AAG CTG GAA AGT GTG GTG
 Gln Ser Ala Phe Ala Gln Ser Glu Pro Glu Leu Lys Leu Glu Ser Val Val
 ATT GTC AGT CGT CAT GGT GTG CGT GCT CCA ACC AAG GCC ACG CAA CTG ATG
 Ile Val Ser Arg His Gly Val Arg Ala Pro Thr Lys Ala Thr Gln Leu Met
 CAG GAT GTC ACC CCA GAC GCA TGG CCA ACC TGG CCG GTA AAA CTG GGT TGG
 Gln Asp Val Thr Pro Asp Ala Trp Pro Thr Trp Pro Val Lys Leu Gly Trp
 CTG ACA CCG CGN GGT GGT GAG CTA ATC GCC TAT CTC GGA CAT TAC CAA CGC
 Leu Thr Pro Arg Gly Gly Glu Leu Ile Ala Tyr Leu Gly His Tyr Gln Arg
 CAG CGT CTG GTA GCC GAC GGA TTG CTG GCG AAA AAG GGC TGC CCG CAG TCT
 Gln Arg Leu Val Ala Asp Gly Leu Leu Ala Lys Lys Gly Cys Pro Gln Ser
 GGT CAG GTC GCG ATT ATT GCT GAT GTC GAC GAG CGT ACC CGT AAA ACA GGC
 Gly Gln Val Ala Ile Ile Ala Asp Val Asp Glu Arg Thr Arg Lys Thr Gly
 GAA GCC TTC GCC GCC GGG CTG GCA CCT GAC TGT GCA ATA ACC GTA CAT ACC
 Glu Ala Phe Ala Ala Gly Leu Ala Pro Asp Cys Ala Ile Thr Val His Thr
 CAG GCA GAT ACG TCC AGT CCC GAT CCG TTA TTT AAT CCT CTA AAA ACT GGC
 Gln Ala Asp Thr Ser Ser Pro Asp Pro Leu Phe Asn Pro Leu Lys Thr Gly
 GTT TGC CAA CTG GAT AAC GCG AAC GTG ACT GAC GCG ATC CTC AGC AGG GCA
 Val Cys Gln Leu Asp Asn Ala Asn Val Thr Asp Ala Ile Leu Ser Arg Ala
 GGA GGG TCA ATT GCT GAC TTT ACC GGG CAT CGG CAA ACG GCG TTT CGC GAA
 Gly Gly Ser Ile Ala Asp Phe Thr Gly His Arg Gln Thr Ala Phe Arg Glu
 CTG GAA CGG GTG CTT AAT TTT CCG CAA TCA AAC TTG TGC CTT AAA CGT GAG
 Leu Glu Arg Val Leu Asn Phe Pro Gln Ser Asn Leu Cys Leu Lys Arg Glu
 AAA CAG GAC GAA AGC TGT TCA TTA ACG CAG GCA TTA CCA TCG GAA CTC AAG
 Lys Gln Asp Glu Ser Cys Ser Leu Thr Gln Ala Leu Pro Ser Glu Leu Lys
 GTG AGC GCC GAC AAT GTC TCA TTA ACC GGT GCG GTA AGC CTC GCA TCA ATG
 Val Ser Ala Asp Asn Val Ser Leu Thr Gly Ala Val Ser Leu Ala Ser Met
 CTG ACG GAG ATA TTT CTC CTG CAA CAA GCA CAG GGA ATG CCG GAG CCG GGG
 Leu Thr Glu Ile Phe Leu Leu Gln Gln Ala Gln Gly Met Pro Glu Pro Gly
 TGG GGA AGG ATC ACC GAT TCA CAC CAG TGG AAC ACC TTG CTA AGT TTG CAT
 Trp Gly Arg Ile Thr Asp Ser His Gln Trp Asn Thr Leu Leu Ser Leu His
 AAC GCG CAA TTT TAT TTG CTA CAA CGC ACG CCA GAG GTT GCC CGC AGC CGC
 Asn Ala Gln Phe Tyr Leu Leu Gln Arg Thr Pro Glu Val Ala Arg Ser Arg
 GCC ACC CCG TTA TTG GAT TTG ATC ATG GCA GCG TTG ACG CCC CAT CCA CCG
 Ala Thr Pro Leu Leu Asp Leu Ile Met Ala Ala Leu Thr Pro His Pro Pro

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 "6289860"

CAA AAA CAG GCG TAT GGT GTG ACA TTA CCC ACT TCA GTA CTG TTT ATT GCC
 Gln Lys Gln Ala Tyr Gly Val Thr Leu Pro Thr Ser Val Leu Phe Ile Ala

 GGA CAC GAT ACT AAT CTG GCA AAT CTC GGC GGC GCA CTG GAG CTC AAC TGG
 Gly His Asp Thr Asn Leu Ala Asn Leu Gly Gly Ala Leu Glu Leu Asn Trp

 ACG CTT CCC GGT CAG CCG GAT AAC ACG CCG CCA GGT GGT GAA CTG GTG TTT
 Thr Leu Pro Gly Gln Pro Asp Asn Thr Pro Pro Gly Gly Glu Leu Val Phe

 GAA CGC TGG CGT CGG CTA AGC GAT AAC AGC CAG TGG ATT CAG GTT TCG CTG
 Glu Arg Trp Arg Arg Leu Ser Asp Asn Ser Gln Trp Ile Gln Val Ser Leu

 GTC TTC CAG ACT TTA CAG CAG ATG CGT GAT AAA ACG CCG CTG TCA TTA AAT
 Val Phe Gln Thr Leu Gln Gln Met Arg Asp Lys Thr Pro Leu Ser Leu Asn

 ACG CCG CCC GGA GAG GTG AAA CTG ACC CTG GCA GGA TGT GAA GAG CGA AAT
 Thr Pro Pro Gly Glu Val Lys Leu Thr Leu Ala Gly Cys Glu Glu Arg Asn

 GCG CAG GGC ATG TGT TCG TTG GCA GGT TTT ACG CAA ATC GTG AAT GAA GCA
 Ala Gln Gly Met Cys Ser Leu Ala Gly Phe Thr Gln Ile Val Asn Glu Ala

 CGC ATA CCG GCG TGC AGT TTG AGA TCT CAT CAC CAT CAC CAT CAC TAA 1323
 Arg Ile Pro Ala Cys Ser Leu Arg Ser His His His His His His End

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 T04220"64E99860

FIGURE 2
pH/Temperature Profile and Stability

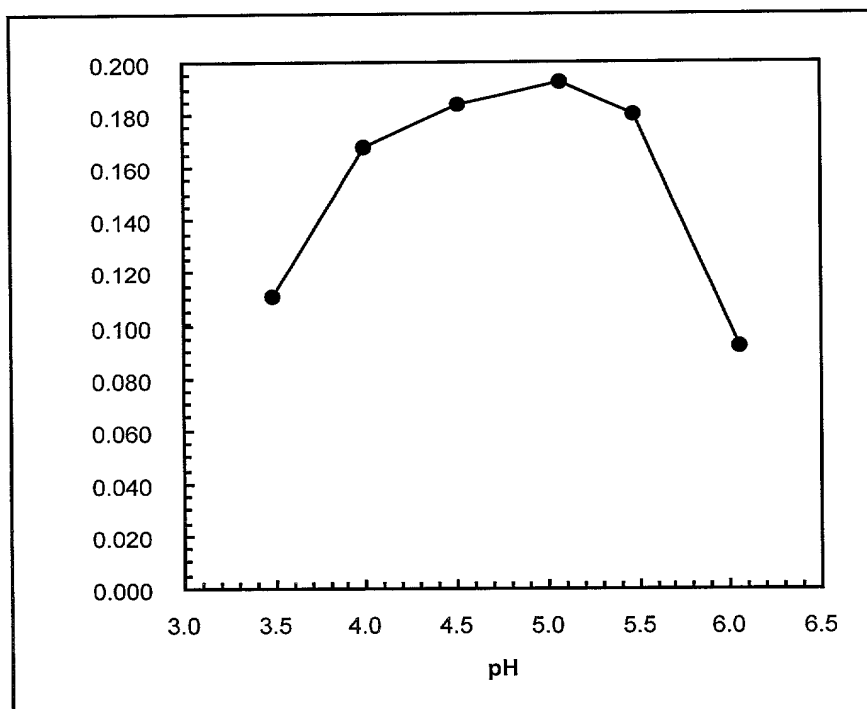
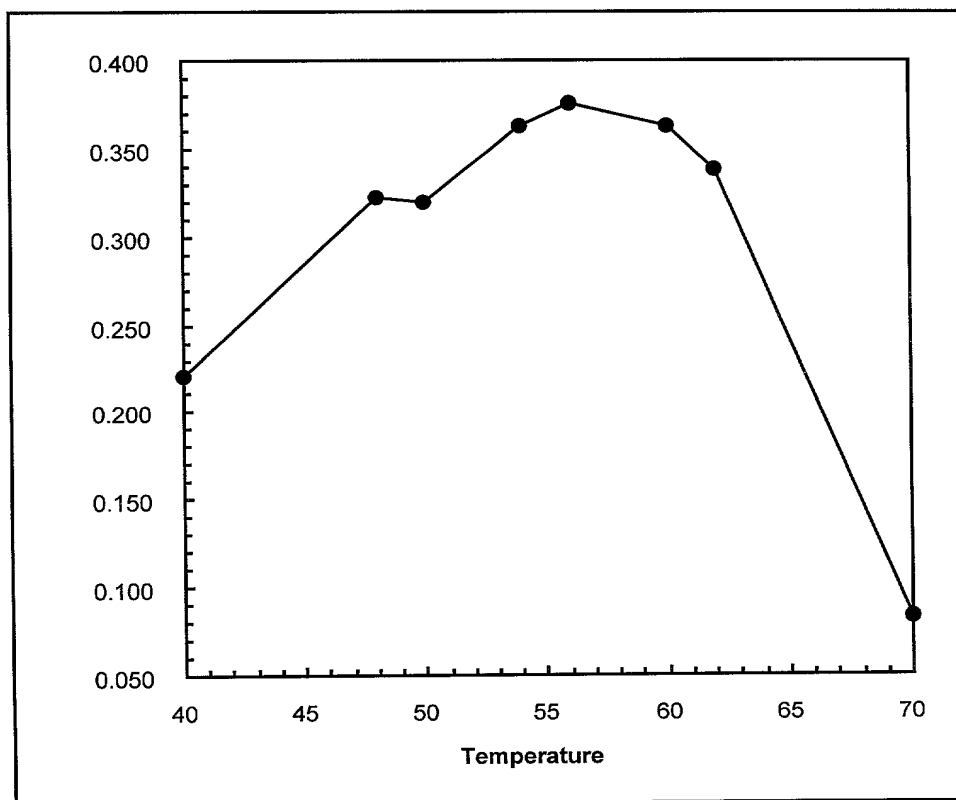
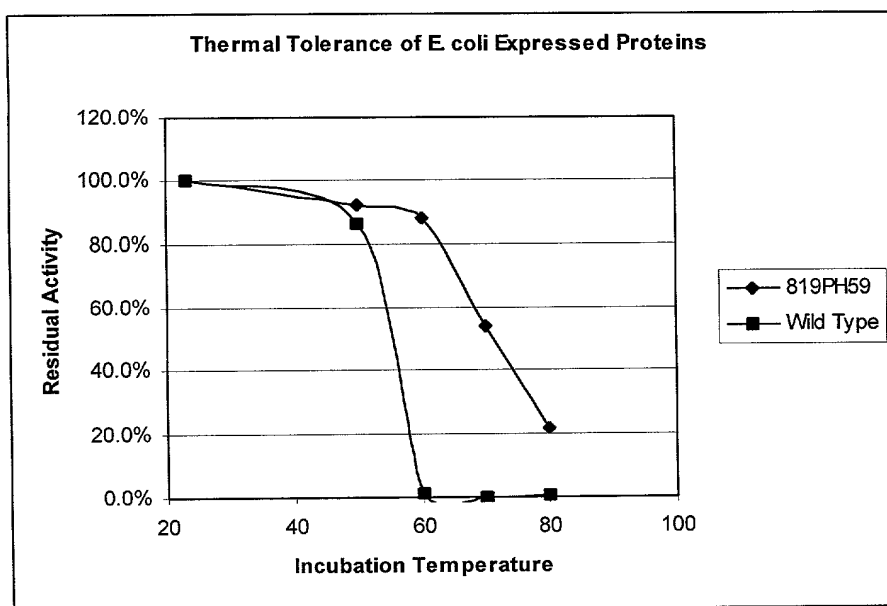


Figure 3:



Downloaded from www.sciencedirect.com

Figure 4

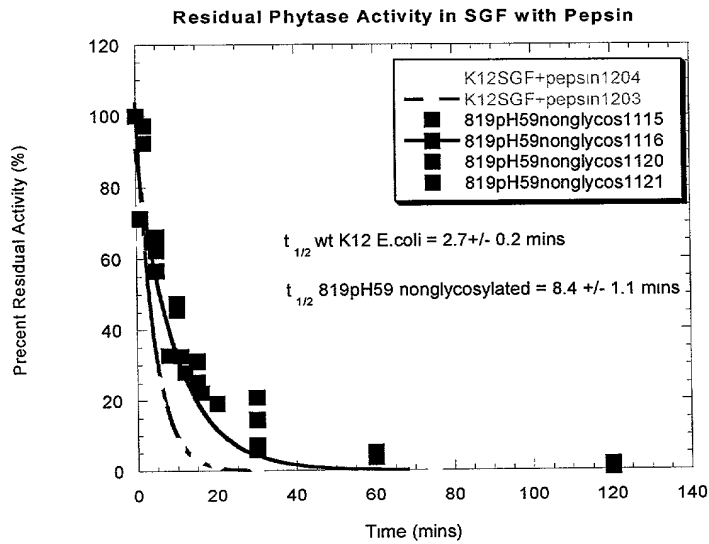


Figure 5:

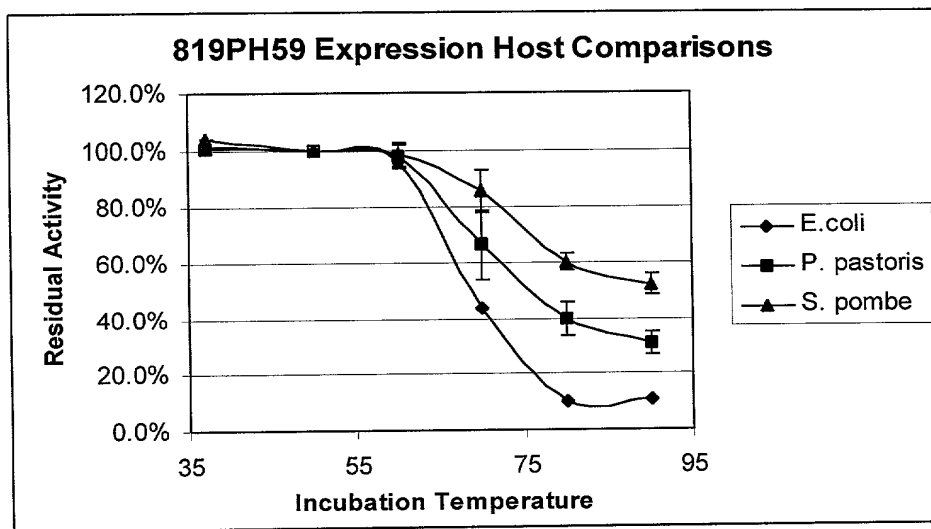


Figure 6.

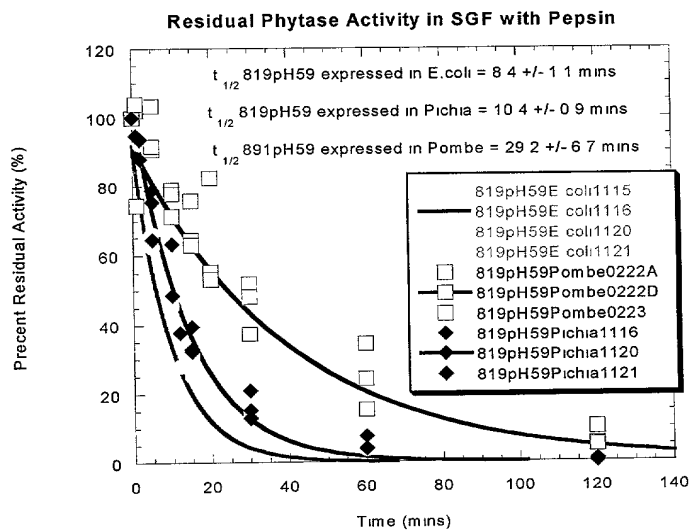


Figure 7a

E. coli appA (GenBank accession no. M58708) (SEQ ID NO:7)

1 taaggagcag aaacaatgtg gtatttactt tggttcgtcg gcattttgtt gatgtgttcg
 61 ctctccaccc ttgtgttggt atggctggac ccgcgtctga aaagttaacg aacgtaggcc
 121 tgatcgccgc cattagcatc gcatcaggca atcaataatg tcagatatga aaagcggaaa
 181 catatcgatg aaagcgatct taatcccatt ttatctctt ctgattccgt taaccccgca
 241 atctgcattc gtcagagtg agccggagct gaagctggaa agtgtggtga ttgtcagtcg
 301 tcatgggtg cgtgctccaa ccaaggccac gcaactgatg caggatgtca cccagacgc
 361 atggccaacc tggccggtaa aactgggttg gctgacaccg cngngtggtg agctaategc
 421 ctatctcgga cattaccaac gccagcgtct ggtagccgac ggattgctgg cgaaaaaggg
 481 ctgcccgcag tctggtcagg tcgcgattat tctgtatgtc gacgagcgta cccgtaaaa
 541 aggcgaagcc ttcgccgccc ggctggcacc tgactgtgca ataaccgtac ataccaggc
 601 agatacgtcc agtcccgatc cgttatttaa tctctaaaa actggcggtt gccaaactgga
 661 taacgcgaac gtgactgacg cgatcctcag cagggcagga gggtaattg ctgactttac
 721 cgggcatcgg caaacggcgt ttcgcgaact ggaacgggtg cttaatttc cgcaatcaaa
 781 cttgtccctt aaacgtgaga aacaggacga aagctgttca ttaacgcagg cattaccatc
 841 ggaactcaag gtgagcgccg acaatgtctc attaacgggt gcgtaagcc tcgcatcaat
 901 gctgacggag atatttctcc tgcaacaagc acagggaatg ccggagccgg ggtggggaag
 961 gatcacgat tcacaccagt ggaacacctt gctaagttg cataacgcgc aattttatt
 1021 gctacaacgc acgccagagg ttgcccgag ccgcgccacc ccgttattag atttgatcaa
 1081 gacagcgttg acgccccatc caccgcaaaa acaggcgat gggtgacat taccacttc
 1141 agtgctgtt atcgccggac acgatactaa tctggcaaat ctggcgggcg cactggagct
 1201 caactggacg ctccccggtc agccggataa cacgccgcca ggtggtgaac ttgtgttga
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 1321 ttacagcag atgcgtgata aaacgccgct gtcattaaat acgccgccc gagaggtgaa
 1381 actgaccctg gcaggatgtg aagagcgaaa tgcgcagggc atgtgtcgt tggcaggtt
 1441 tacgcaaate gtgaatgaag cagcatacc ggcgtgcagt ttgtaatgca taaaaagag
 1501 cattcagtta cctgaatgct ctgaggctga tgacaaacga agaactgtct aatgcgtaga
 1561 ccggaaaagg cgttcacgcc gcatccggcc acittcagtt ttctcttctc tcggagtaac
 1621 tataaccgta atagttag cgttaactgt aagcgggtgt ggcgcgttta atcacaccat
 1681 tgaggatagc gcctttaata ttgacgectg cctgttcag acgtgcatt gacaaactca
 1741 cctctttggc ggtgttcaag ccaaaacgcg caaccagcag gctggtgcca acagaacgcc
 1801 ccacgaccgc ggcatactc accgccagca tcggcggcgt atcgacaatc accagatcgt
 1861 aatggtcgtt cgccattcc agtaattgac gcatccgac g

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Figure 7b

1 taaggagcag aaacaatgtg gtatttactt tggttcgtcg gcattttgtt gatgtgttcg
 61 ctctccccc ttgttttggg atggctggac ccgcgtctga aaagttaacg aacgtaggcc
 121 tgatcgggcg cattagcatc gcatcaggca atcaataatg tcagatatga aaagcggaaa
 181 catatcgatg aaagcgatct taatcccatt tttatctctt ctgattccgt taaccccga
 241 atctgcattc gctcagagtg agccggagct gaagctggaa agtgtggtga ttgtcagtcg
 301 tcatggtgtg cgtgctccaa ccaaggccac gcaactgatg caggatgtca cccagacgc
 361 atggccaacc tggccggtaa aactgggttg gctgacaccg cngggtggtg agctaattcg
 421 ctatctcgga cattaccaac gccagcgtct ggtagccgac ggattgctgg cgaaaaagg
 481 ctgcccgcag tctggtcagg tcgcgattat tgcgatgtc gacgagcgtc cccgtaaaac
 541 aggcgaagcc ttcgccgccg ggctggcacc tgactgtgca ataaccgtac ataccaggc
 601 agatacgtcc agtcccgtc cgttatttaa tctctaaaa actggcgtt gccaaactgga
 661 taacgcgaac gtgactgacg cgatcctcag cagggcagga gggtaattg ctgacttac
 721 cgggcatcgg caaacggcgt ttcggaact ggaacgggtg cttattttc cgcaatcaa
 781 cttgtgcctt aaacgtgaga aacaggacga aagctgttca ttaacgcagg cattaccatc
 841 ggaactcaag gtgagcgccg acaatgtctc attaacgggt gcggtgaagc tcgcatcaat
 901 gctgacggag atatttctc tgcaacaagc acagggaatg ccggagccgg ggtggggaag
 961 gatcaccgat tcacaccagt ggaacacctt gctaagttg cataacgcgc aattttatt
 1021 gctacaacgc acgccagagg ttgccgcag ccgcgccacc ccgttattag atttgatcaa
 1081 gacagcgttg acgccccatc caccgcaaaa acaggcgtat ggtgtgacat taccacttc
 1141 agtgcgtttt atcgccggac acgatactaa tctggcaaat ctggcgggcg cactggagct
 1201 caactggacg ctccccgtc agccggataa cacgccgcca ggtggtgaac tgggtttga
 1261 acgctggcgt cggctaagcg ataacagcca gtggattcag gtttcgctgg tctccagac
 1321 ttacagcag atgcgtgata aaacgccgt gtcattaaat acgccgccc gagaggtgaa
 1381 actgaccctg gcaggatgtg aagagcgaaa tgcgcagggc atgtgtcgt tggcaggtt
 1441 tacgcaaata gtgaatgaag cagcatacc ggctgcagt ttgtaatgca taaaaagag
 1501 cattcagtta cctgaatgct ctgaggctga tgacaaacga agaactgtct aatgcgtaga
 1561 ccggaagagg cgttcacgcc gcacccggcc actttcagtt ttctcttc tcggagtaac
 1621 tataaccgta atagttatag ccgtaactgt aagcgggtct ggccggtta atcacacat
 1681 tgaggatagc gcctttaata ttgacgcctg cctgttcag acgtgcatt gacaaactca
 1741 cctctttggc ggtgttcaag ccaaacgcg caaccagcag gctggtgcca acagaacgc
 1801 ccacgaccgc ggcatactc accgccagca tcggcggcgt atcgacaatc accagatcgt
 1861 aatggtcgtt cgccattcc agtaattgac gcacccgac g

0986379.052401
 104250"6499860

Figure 8

Amino acid sequence for E. coli appA (wild type) (SEQ ID NO:8)

MKAILIPFLSLLIPLTPQSAFAQSEPELKLESVVIVSRHGVRAPTKATQLMQDVT
 PDAWPTWPVKLGWLTPRGGELIAYLGHYQRQRLVADGLLAKKGCPQSGQV
 AIIADVDERTRKTGEAFAAGLAPDCAITVHTQADTSSPDPLFNPLKTGVCQLD
 NA
 NVTDAILSRAGGSIADFTGHRRQTAFRELERVLNFPQSNLCLKREKQDESCSLT
 QALPSELKVSADNVSLTGAVSLASMLTEIFLLQQAQGMPEPGWGRITDSHQW
 NTLLSLHNAQFYLLQRTPEVARSRATPLLDLIKALTTPHPPQKQAYGVTLPSTV
 LFIAGHDTNLANLGGALELNWTLPGQPDNTPPGGELVFERWRRLSDNSQWIQ
 VSLVFQTLQQMRDKTPLSLNTPPGEVKLTLAGCEERNAQGMCSLAGFTQIVN
 EARIPACSL

Bold-Underlined amino acid residues are shown below in the modified appA enzyme
 (SEQ ID NO:10)

MKAILIPFLSLLIPLTPQSAFAQSEPELKLESVVIVSRHGVRAPTKATQLMQDVT
 PDAWPTWPVKLGELTPRGGELIAYLGHYWRQRLVADGLLPKCGCPQSGQVA
 IIAADVDERTRKTGEAFAAGLAPDCAITVHTQADTSSPDPLFNPLKTGVCQLDN
 A
 NVTDAILEEAGGSIADFTGHYQTAFRELERVLNFPQSNLCLKREKQDESCSLTQ
 ALPSELKVSADCVSLTGAVSLASMLTEIFLLQQAQGMPEPGWGRITDSHQWN
 TLLSLHNAQFDLQRTPEVARSRATPLLDLIKALTTPHPPQKQAYGVTLPSTVLF
 IAGHDTNLANLGGALELNWTLPGQPDNTPPGGELVFERWRRLSDNSQWIQVS
 LVFQTLQQMRDKTPLSLNTPPGEVKLTLAGCEERNAQGMCSLAGFTQIVNEA
 RIPACSL

T04250" 6429860